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THE CONCENTRATION OF CERTAIN TRACE ELEMENTS IN THE WOOL OF SHEEP WITH FLUOROSIS

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ABSTRACT: The aim of this study was to evaluate the trace element levels in the wool of sheep, with and without fluorosis, living in a volcanic area of Turkey. Fifteen Akkaraman sheep with fluorosis in the Agri region, to the north of Lake Van, and 10 Akkaraman sheep without fluorosis in the Van region, to the south of Lake Van in the eastern part of Turkey, were investigated. The urinary fluoride levels were measured with an ion selective electrode. The sheep with fluorosis were identified by clinical examination and the presence of a high urinary fluoride level. The wool samples were obtained from the neck region. The trace element concentrations in these samples were measured using an atomic absorption spectrophotometer. It was determined that in the fluorosis group, compared to the control group, the copper and zinc levels were significantly decreased ($p \leq 0.05$), and a non-significant decrease ($p \geq 0.05$) was present for the levels of the nickel, manganese, iron, and cobalt.

Keywords: Akkaraman sheep; Fluorosis; Trace elements; Wool.

INTRODUCTION

Fluoride (F), the ion of the highly electronegative element fluorine, is a potential environmental hazard. The main sources of F intake are usually water and nutrients, while generally very low levels of F are found in the air. F belongs to the halogen group of elements and is found naturally in water, soil, animals, and plants. The prevalence of F in large quantities in nutrients and drinking water in the environment often leads to adverse health effects. Fluorosis may become a serious public health issue when drinking water contains more than 1–1.5 ppm of fluoride. Fluoride-containing oral healthcare products may be a risk factor for fluoride intoxication in childhood.[1-5] Various studies have been conducted on fluoride and its relation to health and the environment. F intake by humans generally occurs through the intake of food and water since air usually contains low levels of F. An excessive intake of fluoride (F) is known to cause a wide range of adverse health effects along with damage to bones and teeth.[6-10]

Trace elements are inorganic substances that play a role in catalytic, enzymatic, and structural activities in several important activities in the organism and should be taken exogenously via nutrients and water. The trace elements that enter the organism bond with various blood proteins and are dispersed into all tissues. Trace element quantities are closely associated with various factors such as nutrition, age, diseases, and ecology.[11]

Although previous research has studied the effects of fluorosis on the serum of sheep[12-15] and cattle[16,17] and the trace element levels in their tissues,[18,19] no data were found on the impact of fluorosis on the trace element levels in their wool and hair. Therefore, the aim of present study was to determine the trace element levels in the wool of sheep with chronic fluorosis.

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MATERIALS AND METHODS

Animal material: The sheep with fluorosis were chosen from Ayranci village of Dogubeyazit, Agri, Turkey, where fluorosis was endemic. Fifteen two-year-old or older Akkaraman sheep with fluorosis were selected to provide the wool samples from sheep with fluorosis. The signs of fluorosis were evaluated through the clinical examination of the teeth and joints in order to determine the presence and history of fluorosis. The urinary F levels of the animals were also determined. For the control group of sheep without fluorosis, ten Akkaraman sheep of same age from Van province located south of Agri were selected in whom the absence of fluorosis was determined through clinical and laboratory examination.

Figure 1. Location of the sheep, with and without fluorosis, in Agri and Van Provinces, Turkey.

Determination of urinary fluoride levels: Urine samples were obtained from several sheep, with procedures similar to those explained in previous research by the authors.[20] The urine samples, obtained in accordance with these procedures, were transferred into polyethylene tubes. The urinary fluoride levels were measured immediately with a WTW PH / ION 738 brand ion meter using an ion-selective electrode.[21]

Collection and preparation of the wool samples: The wool samples were obtained from the neck region of the healthy sheep and the sheep with fluorosis and washed 4 times with 1% Triton™ X-100 solution. Subsequently, they were laved twice with deionized water and then rinsed. The samples were drained and dried at 100ºC for 2 hours. After this step, 200 mg samples were weighed on a precision scale and transferred to the test tubes. 1.2 mL of nitric acid/perchloric acid (1/5) mixture was added to each tube. The samples placed in the tubes were stored at 60ºC for 6 to 7 hours. Then, 1% Triton™ X-100 was added to the solute samples to obtain 10 mL. Analysis of these samples was conducted with an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 800).[22]

Statistical analysis: The findings were statistically analysed (student T-test) and the results were regarded as significant at $p<0.05$ (SPSS 22.0).

RESULTS

The urinary fluoride levels of the control and fluorosis group sheep are presented in Table 1 and the wool trace element content is presented in Table 2.

Table 1. Urinary fluoride levels in sheep from the control and fluorosis groups[20]

Parameter	Control group (n=10)	Fluorosis group (n=15)
Urinary fluoride level (ppm)	1.50±0.30	6.74±0.49*

*$p \leq 0.001$ in comparison to the control group.

Table 2. Trace element levels in the wool of sheep from the control and fluorosis groups

Parameter	Control group (n=10)	Fluorosis group (n=15)	p value
Nickel (mg/L)	0.142±0.046	0.139±0.041	0.882
Copper (mg/L)	0.090±0.027	0.062±0.018*	0.007
Manganese (mg/L)	0.348±0.124	0.333±0.163	0.932
Iron (mg/L)	0.694±0.375	0.675±0.352	0.897
Zinc (mg/L)	3.347±0.712	2.694±0.611*	0.029
Cadmium (mg/L)	0.008±0.001	0.007±0.001	0.997
Cobalt (mg/L)	0.040±0.008	0.033±0.018	0.242

*$p \leq 0.05$ in comparison to the control group.

As presented in Table 1, a highly significant difference was observed in the urinary F levels in the groups. The mean urinary F levels of the sheep with fluorosis was 4.5 times higher than that of the control group without fluorosis.

It was determined that in the fluorosis group, compared to the control group, the copper and zinc levels were significantly decreased ($p \leq 0.05$), and a non-significant decrease ($p \geq 0.05$) was present for the levels of the nickel, manganese, iron, and cobalt.

DISCUSSION

Fluorosis is a chronic toxicity due to prolonged exposure to high fluoride levels and is a serious problem in ruminant animals.[23] The problem is observed due to elevated

F levels in drinking water in several countries.[24] Volcanic areas, especially, around the world may have an elevated F content in drinking water. High F levels have been reported in drinking water in Turkey, especially in regions located around the volcanic mountain, Tendurek.[25]

The normal F level in sheep urine is between 1.00 and 1.40 ppm. In the present study, the mean urine F level in the sheep with fluorosis was determined as 6.74±0.49 ppm, and such level is consistent with the results obtained by Altintas et al. for sheep in endemic F areas due to natural F sources.[4] The urinary F levels determined in the control group in the present study (1.50±0.30 ppm) are also consistent with the values reported in literature.[20]

Animals such as sheep, goat, and cattle are more susceptible to fluorosis in comparison to other animals. In contrast, pigs and horses are partially resistant, while poultry are completely resistant to the disease. Serum, wool, hair, liver, and kidney have been used to determine the mineral levels in animal tissues. However, it was reported that the measurement of tissue, hair, or wool mineral levels led to more accurate results when compared to serum analysis in animals.[4] Although serum,[15,26,27] bone,[19,28] and tissue[18,29] trace element levels were extensively studied in literature, previous studies did not investigate the trace element levels in the wool of sheep with fluorosis.

Zinc and copper are the cofactors of several enzymes that play significant roles in growth, immune system, cell respiration, redox reactions, and protein synthesis. Cu and Zn are also important cofactors of the superoxide dismutase (SOD) enzyme, which is an important component in the antioxidant system, and plays a role in the alleviation and termination of peroxidation of free radicals.[30]

Varied findings were reported for zinc levels in fluorosis and it was reported that decreases were observed in serum,[26,27,31] bone,[19,28] liver,[28,32,19] testis, plasma,[32] and kidney,[19,32] and increases were observed in bone[32] and kidney levels.[29] The present study demonstrated decreased zinc levels in the wool of sheep with chronic fluorosis.

It was found that fluoride reduced copper levels in rat bones,[29,30] in sheep serum,[15] teeth and gums,[19] and in human serum.[26,27] In the present study, the low copper levels determined in the sheep with fluorosis was consistent with the reports in the literature.

Bharti et al.[34] examined fluoride-induced oxidative stress and demonstrated that fluoride administration significantly reduced the SOD activity. The low Cu levels in the sheep with fluorosis, determined in the present study, could be associated with fluoride-induced oxidative stress. The increased use of Cu and Zn to counteract fluoride-induced oxidative stress could be responsible, at least partially, for the decrease in levels of these elements in blood.

It was reported that the serum Fe,[31] Mn,[31] and Ni[12,24,31] levels were significantly decreased in ruminants. In contrast,[17] it was established that fluoride increased serum Mn levels in cattle. In the present study, it was observed that the wool nickel, manganese, iron, and cobalt levels did not change significantly.

CONCLUSIONS

In conclusion, the present study explicitly demonstrated that serum Cu and Zn levels decreased in sheep with chronic fluorosis. Hence, these minerals should be supplemented in sheep with fluorosis. It could be suggested that the decrease in the trace element content in wool, which is an important indicator of overall mineral levels, occurred due to the decrease of particular trace elements, namely copper and zinc.

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